the protein-lipid interface to drive activation of the voltage-sensors. This motif is an important pharmacological target for amphipathic neurotoxins and it has been suggested that it is conserved in other voltage-gated ion channels. Here we show that the four S3b-S4 paddle motifs within the T-type calcium channel could be transplanted into four-fold symmetric Kv channel to individually examine their contributions to the kinetics of voltage sensor activation and pharmacology. Using these chimeric constructs, we screened existing gating-modifier toxins against the putative paddle motif from each domain of T-type calcium channel, Cav3.1. We found that the four individual paddle motifs of Cav3.1 channels display unique toxin binding capabilities, suggesting that gating-modifier toxins can bind to T-type calcium channels in a domain-specific fashion. Comparing ProTx-II-like toxins effect on T-type calcium channels and chimeras suggests potential amino acids involved in the direct interaction between toxin and channels.

2922-Pos Board B352

Engineering Selectivity in RGK Protein Inhibition of Cav1/Cav2 Channels

Akil Puckerin.
Columbia University, New York, NY, USA.

High-voltage activated calcium channels ($Ca_V1.1$–$Ca_V1.4$; $Ca_V2.1$–$Ca_V2.3$) link electrical signals to vital physiological responses in excitable cells. Molecules that block Ca_V1/Ca_V2 channels are important therapeutics. Rad/Rem/Rem2/Gem (RGK) proteins are small Ras-like G-proteins that potently and indiscriminately inhibit all Ca_V1/Ca_V2 channels. The practical utility of RGKs as genetically-encoded Ca_V channel blockers would be vastly improved if it were possible to engineer versions that display selective inhibition of distinct Ca_V1/Ca_V2 isoforms. Ca_V1/Ca_V2 pore-forming α_1 subunits require binding to auxiliary $Ca_V\beta$s to generate functional channels. All RGKs bind wild-type (wt) $Ca_V\beta$ and this interaction is disrupted in a mutated $Ca_V\beta$ ($Ca_V\beta_{TM}$). We compared the ability of Rem and Gem to inhibit four distinct Ca_V1s ($Ca_V1.2$, $Ca_V1.3$, $Ca_V2.1$ and $Ca_V2.2$) reconstituted with either $wtCa_V\beta$ or $Ca_V\beta_{TM}$ in HEK293 cells. While both Rem and Gem blocked all channels reconstituted with $wtCa_V\beta$, Rem uniquely suppressed $Ca_V1.2+\beta_{2aTM}$ channels, a signature of $Ca_V\beta$-binding-independent inhibition. Using FRET analyses, chimeric Rem/Gem proteins, and electrophysiology we show that $Ca_V\beta$-binding-independent inhibition of $Ca_V1.2$ involves direct interaction of Rem C-terminus with $Ca_V1.2\alpha_{1C}$ N-terminus, and additionally requires the Rem nucleotide-binding domain. A mutant Rem that no longer interacts with $Ca_V\beta$ ($Rem_{\beta\text{-null}}$) selectively inhibited $Ca_V1.2+wtCa_V\beta$ channels. We further profiled the prevalence of β-binding-dependent and -independent mechanisms of inhibition by Rem2 and Rad across Ca_V1/Ca_V2 channel families. While Rem2 relied on $Ca_V\beta$ binding to inhibit all four Ca_Vs tested, Rad displayed β-binding-independent inhibition of $Ca_V1.2$ and $Ca_V2.2$. Consistent with this, $Rem_{\beta\text{-null}}$ selectively inhibited $Ca_V1.2$ and $Ca_V2.2$ channels reconstituted with $wtCa_V\beta$. In summary, our results have revealed a latent capability of distinct RGK proteins to block particular Ca_V1/Ca_V2 channels in an α_1-subunit-specific manner. We have exploited this feature to generate genetically-encoded Ca_V-isoform-selective inhibitors.

2923-Pos Board B353

Ventricular L-Type Ca^{2+} Channels and Expression of RGK Proteins in Mouse Models Associated with Diabetes

Jessica Köth, Christian Fabisch, Stefan Herzig, Jan Matthes.
Department of Pharmacology, University of Cologne, Cologne, Germany.

Background: In a diabetic mouse model (db/db) we have shown reduced I_{CaL} density with unchanged single-channel activity and reduced expression of the LTCC pore $Ca_v1.2$ (Pereira et al., Diabetes 2006;55:608-15). Of note, LTCC expression and function can be decreased by RGK proteins, including the diabetes-associated protein Rad. **Aim of the study:** In the present study, we investigate the association between cardiac Rad expression with the expression and function of ventricular LTCC in two mouse models with diabetes-related metabolic disturbances (leptin-deficient obese ob/ob mice and insulin receptor substrate 2 deficient IRS2-k.o. mice). **Methods:** We obtained expression of Rad and $Ca_v1.2$ protein (Western-blot) and mRNA (qRT-PCR) in murine ventricles and recorded whole-cell I_{CaL} in freshly isolated ventricular myocytes. **Results:** The only significant change at the mRNA level was an increased Rad expression in IRS2-k.o. mice at 16 weeks of age (206 ± 17%). In line with this finding I_{CaL} density was significantly decreased (IRS2-k.o.: −7.8 ± 0.8 pA/pF; wildtype: −10.9 ± 0.9 pA/pF). At an age of 28 weeks, we found expression of both Rad and $Ca_v1.2$ protein to be significantly increased in ventricles from ob/ob mice compared to age-matched widtypes (273 ± 23% and 159 ± 14%, respectively) while I_{CaL} density was unchanged (ob/ob: −8.4 ± 0.4 pA/pF; wildtype: −8.9 ± 0.5 pA/pF). **Summary and discussion:** Our data support the idea of Rad being involved in regulation of I_{CaL} in diabetes. Regarding I_{CaL} Rad seems to act either causal (I_{CaL} decrease in IRS2-k.o. at 16 weeks) or compensatory (increased $Ca_v1.2$ expression but unchanged I_{CaL} in ob/ob at 28 weeks). Differences observed between the two investigated diabetic mouse models might be explained by differences in the underlying pathomechanisms (lack of IRS2 vs. leptin deficiency, respectively).

2924-Pos Board B354

Inhibition of Human $Ca_V2.3$ Channels via μ-, δ- and κ-Opioid Receptor Activation

Geza Berecki, Leonid Motin, **David J. Adams**.
Health Innovations Research Institute, RMIT University, Melbourne, Australia.

Neuronal voltage-gated $Ca_v2.3$ channels are widely expressed in the central and peripheral nervous system where they contribute to neurotransmission and pain sensation. However, modulation of the $Ca_v2.3$ channel through G protein-coupled (GPC) μ- and δ-opioid receptors is poorly defined and has not previously been reported for κ-opioid receptors. We hypothesized that activation of human μ-, δ- or κ-opioid receptors modulates $Ca_v2.3$ channels via G protein signaling. Whole-cell Ba^{2+} currents were recorded in HEK293T cells co-expressing human $Ca_v2.2$ or $Ca_v2.3$ channels and μ-, δ- or κ-opioid receptors. Selective opioid receptor agonists and antagonists were used to study receptor modulation. The involvement of intracellular signaling pathways was investigated using specific inhibitors of GPC receptor-G protein coupling. Activation of μ-, δ- or κ-opioid receptors inhibited $Ca_v2.3$ and $Ca_v2.2$ channel current amplitude by ~45% and ~60%, respectively. Inhibition of $Ca_v2.3$ was not dependent on the type of subunit co-expressed. Inhibition of the $Ca_v2.3$ channel was primarily voltage independent, as depolarizing prepulses could not relieve the inhibited current. This was in marked contrast with the primarily voltage-dependent modulation of $Ca_v2.2$ channels that showed nearly complete recovery of the inhibited current with depolarizing prepulses. For all three types of opioid receptors, the pathway leading to $Ca_v2.3$ channel inhibition was sensitive to pertussis toxin and intracellular application of GDP-β-S. Similarly, the overexpression of a G protein subunit scavenger, myristoylated-phosducin, significantly reduced the magnitude of $Ca_v2.3$ channel inhibition. Here we demonstrate that $Ca_v2.3$ channels are efficiently inhibited by activation of μ-, δ- or κ-opioid receptors. Inhibition occurs via voltage-independent G protein signaling mechanisms. These results suggest opioid receptor controls specific members of the Ca_v2 channel family via differential signaling pathways. Neuronal $Ca_v2.3$ channels are therefore potential targets for opioid analgesics.

2925-Pos Board B355

Control of Functional Targeting of Cav1.2 Channels by the γ6

Roman Shirokov, Thomas Comollo, Rose Rendon.
Pharmacology and Physiology, NJMS, Newark, NJ, USA.

γ6 subunit of voltage-gated calcium channels is expressed in the heart and in the brain. It modulates gating of T-type channels (Hansen et al., 2004; Lin et al., 2008). It also associates with CaV1.2 channels and enhances their inactivation in the presence of the β1b, but not β2b, subunit (Yang et al., 2010). We found that γ6 subunit dramatically reduces the number of functional α1/β2a channels expressed in tsA-201 cells. Channels with the γ6 are trapped in the Golgi complex. Deletions of N-termini of α1 and γ6 subunits restore functional targeting to the plasma membrane.

We propose that the γ6 regulates functional expression of CaV1.2 channels by interacting with the pore-forming α1 subunit or with another protein in the complex.

2926-Pos Board B356

L-Type Ca^{2+} Channel Cavb Subunits Associate with and Differentially Regulate the Cardiac Cav3.2 T-Type Ca^{2+} Channel Currents

Marites T. Woon[1], Ravi C. Balijepalli[2].
[1]University of Wisconsin, Madison, Madison, WI, USA, [2]Medicine, University of Wisconsin, Madison, Madison, WI, USA.

Low voltage activated T-type calcium channels (TTCC) play a pivotal role in the developing heart. Although the TTCC isoforms, $Ca_v3.1$ and $Ca_v3.2$, underlie cardiac TTCC current ($I_{Ca,T}$) and are expressed in atrial and ventricular myocytes during development, their expression and roles recede in the adult heart. However, previous studies have demonstrated the re-expression of $I_{Ca,T}$ in pathological cardiac hypertrophy, suggesting that TTCCs contribute to the altered Ca^{2+} cycling and signaling in these pathological conditions. In addition, the reported altered expression of some $Ca_v\beta$ subunits (specifically β_1 and β_2 subunits) of the high voltage activated L-type calcium

channels (LTCC) has been reported in hypertrophy and heart failure. We hypothesize that the altered expression of the $Ca_v\beta$ subunits in cardiac hypertrophy and heart failure results in the altered coupling and regulation of $I_{Ca,T}$. We found an increase in the $Ca_v\beta_1$ and $Ca_v\beta_3$ mRNA and $Ca_v\beta_2$ and $Ca_v\beta_4$ subunit isoform at the protein level in mouse ventricles in a transthoracic aortic constriction (TAC) induced pathological cardiac hypertrophy model. Whole-cell patch clamp electrophysiology using transiently transfected HEK293 cells revealed that co-expression of $Ca_v\beta_1$ or $Ca_v\beta_2$ with $Ca_v3.2$ channel isoform resulted in a significant increase in peak $I_{Cav3.2}$ and a rightward shift in the $V_{1/2}$ of activation and significantly slower inactivation of $I_{Cav3.2}$. On the other hand, co-expression of $Ca_v\beta_3$ or $Ca_v\beta_4$ significantly reduced the peak $I_{Cav3.2}$. In contrast, co-expression of $Ca_v\beta$ isoforms did not alter $I_{Cav3.1}$. Furthermore, co-immunoprecipitation studies in transiently transfected HEK293 cells also demonstrated that the $Ca_v3.2$ channel separately co-immunoprecitated with anti-$Ca_v\beta_1$ or anti-$Ca_v\beta_2$ antibody. In conclusion, our data suggest that the $Ca_v\beta_1$ and $Ca_v\beta_2$ subunits of the LTCC may regulate $I_{Cav3.2}$ in cardiomyocytes during pathological cardiac hypertrophy.

2927-Pos Board B357

Homologous Serine/Threonine in the Ca_V 2.2α_1 and 2.3α_1 Subunits Behave Similarly, as Stimulatory and Inhibitory PKC Sites

Ganesan L. Kamatchi.
Biology, Norfolk State University, Norfolk, VA, USA.

High voltage-gated calcium (Cav) channels are regulated by PKC isozymes. These isozymes target selected serine/threonine (Ser/Thr) PKC phosphorylation sites in the intracellular regions of Cavα_1 subunits of these channels. It has been found earlier using Cav2.2α_1 subunits that stimulatory (Thr-422, Ser-2108 and Ser-2132) and inhibitory (Ser-425) PKC sites exist and their activation with PKC isozymes led to potentiation and depression of calcium currents (I_{Ca}) respectively. Based on the above report, it was planned to examine if the homologous sites in the Cav2.3α_1 subunits behave similarly. In this regard the WT Cav2.3α_1 or Ser/Thr Ala mutants of stimulatory (Thr-365, Ser-1995 and Ser-2011) or inhibitory (Ser-369) sites were expressed along with β_{1b} and $_2$/δ cDNA subunits in *Xenopus* oocytes and the barium currents (I_{Ba}) were studied. Intracellular injection of PKC isozymes βII or ε potentiated WT Cav2.3 currents. While both PKC βII and ε potentiated I_{Ba} through Thr-365 (**T365**/S369A/S1995A/S2011A), only PKC ε increased I_{Ba} through Ser-1995 (T365A/S369A/**S1995**/S2011A) channels. Ser-2011 failed to act as a stimulatory site contrary to its homologous site, Ser-2132 in the Cav2.2α_1 subunits. However, Ser-369 acted as inhibitory site as its homolog Ser-425 in the Cav2.2α_1 subunits. Both PKC βII and ε inhibited I_{Ba} through Ser-369 (T365A/**S369**/S1995A/S2011A) channels. When both Thr-365 and Ser-369 were present (**T365/S369**/S1995A/S2011A), I_{Ba} was neither stimulated nor inhibited. However, stimulation was dominant when two stimulatory sites (Thr-365 & Ser-1995) were present along with Ser-369. Experiments with other mutants, including Ser/Thr Asp constructs are being studied and will be discussed.

2928-Pos Board B358

Norepinephrine Upregulates T-Type Calcium Channels in Rat Pinealocytes

Haijie Yu[1], Jong Bae Seo[1], Seung-Ryoung Jung[1], Duk-Su Koh[1,2], Bertil Hille[1].
[1]Physiology and Biophysics, University of Washington, Seattle, WA, USA, [2]Physics, Pohang University of Science and Technology, Pohang, Korea, Republic of.

The mammalian pineal has a circadian rhythm of melatonin secretion at night triggered by norepinephrine (NE) released from sympathetic nerve terminals. We asked whether functional expression of voltage-gated calcium channels in rat pinealocytes is changed by culturing them in NE as a surrogate for the night signal. Channel activity was assayed as ionic currents under patch clamp. Cultured without NE, pinealocytes showed only non-inactivating L-type dihydropyridine-sensitive calcium current. After 24 h in NE, an additional low-voltage activated transient calcium current developed whose pharmacology and kinetics corresponded to a T-type channel. This change was initiated by β-adrenergic receptors, cyclic AMP, and protein kinase A as revealed by pharmacological experiments. Quantitative PCR experiments showed mRNA for Cav1.4 (L-type), Cav3.1 and Cav3.2 (both T-type). Cav3.1 T-type channel mRNA was significantly elevated by culture in NE, but those for Cav1.4 and Cav3.2 were not. After only 8 h of NE treatment, Cav3.1 mRNA was already elevated, but the transient calcium current was not. Even a 16 h wait without NE following the 8 h NE treatment induced little additional transient current. However, these cells were primed to make transient current after a second NE exposure. Induction of transient current was sensitive to the inhibitors of intracellular protein trafficking. The NE-induced T-type channel mediated an increased calcium entry during short depolarizations and supported modest transient electrical responses to depolarizing stimuli. Such experiments reveal a potential for circadian regulation of pinealocyte electrical excitability and calcium signaling. This work is supported by National Institutes of Health grants GM-83913, NS-08174 and DK-080840.

2929-Pos Board B359

Single-Channel Analysis of the Inhibition of the Calcium Dependent Inactivation by the C-Terminal Modulator Domain of Cav1.3 Channels

Elza Kuzmenkina, Elena Novikova, Wanchana Jangsangthong, Stefan Herzig.
Pharmacology, University of Cologne, Cologne, Germany.

Cav1.3 channels belong to the family of the voltage-gated L-type calcium channels. Because of activation at low voltage thresholds, Cav1.3 channels are involved in regulation of the cell firing but they are also linked to the generation of the oxidative stress. Calcium-dependent inactivation (CDI) is a negative feedback process terminating the potentially toxic calcium influx (Johny et al., Nat Commun 2013;4:1717). Multiple Cav1.3 splice isoforms, which can be simultaneously expressed in the same tissue, differ in their channel gating and CDI (Bock et al., J Biol Cem 2011;286:42736).

We applied single-channel patch-clamp measurements to compare two native isoforms with alternatively spliced C-terminus. Cav1.3 42 isoform has a long C-terminus containing C-terminal modulator domain (CTM), whereas in the short C-terminus of Cav1.3 42A isoform CTM is truncated. CTM shifts channel activation to higher voltages and inhibits CDI.

Here, we observed that calcium influx from a few single-channel openings lead to CDI, visible as a decay of the average single-channel current. However, the extent of CDI was significantly reduced in Cav1.3 42 isoform as compared with 42A isoform. Furthermore, we observed the shortening of the open times as a result of CDI (Imredy and Yue, Neuron 1994;12:301). The degree of the open-time shortening was dependent on the prior calcium influx (Josephson et al., J Physiol 2010;588:213) with a steeper dependence for Cav1.3 42A isoform.

Additionally, we performed experiments with a channel agonist S(-)BayK 8644. The boosting of the channel activity by BayK 8644 eliminated the difference between the isoforms, resulted in a high extent of CDI for both splice variants.

Our findings suggest the ability of CTM to inhibit CDI depends on the channel activity, which can be in turn modified by CTM.

2930-Pos Board B360

Serum Factor Alters T-Type Cav3.2 Gating Kinetics and Current Density

Gray Evans, Slobodan M. Todorovic.
Department of Anesthesiology, University of Virginia, Charlottesville, VA, USA.

T-type Calcium channels play a critical role in regulating neuronal excitability and modulating sensory transmission. The Cav3.2 channel isoform is highly expressed in peripheral nociceptors as well as in the pain-processing regions of the dorsal horn of the spinal cord. Augmenting Cav3.2 currents has been shown to induce hyperexcitability in nociceptive neurons in vitro and hyperalgesia in vivo. These studies strongly suggest that potentiation of the Cav3.2 channel results in abnormal nociceptive transmission, which could contribute to a variety of clinical pain syndromes. Therefore, it is important to identify endogenously produced molecular species that modulate Cav3.2 currents. Using the patch-clamp technique and stably transfected human embryonic kidney cells (HEK-293) expressing the Cav3.2 channel, we have begun to characterize a factor found in fetal bovine serum (FBS) that profoundly affects Cav3.2 channel gating kinetics. Specifically, when compared to baseline recombinant currents, 1% serum produces maximal increases in current magnitude (350%; $p<.001$), conductance (150%, $p<.001$), rate of macroscopic inactivation (47.1%; $p<.001$) and deactivation (74.3%; $p<.001$). Furthermore, 1% serum induces a hyperpolarizing shift in voltage-dependence of activation (V50) (−4.77mV; $p<.001$) with minimal effect on voltage-dependence of inactivation. In contrast, we found that recombinant CaV3.1 currents were completely insensitive. Similar to recombinant Cav3.2 currents, T-currents from rat dorsal root ganglia (DRG) cells exhibited comparable changes, with 1% serum producing maximal increases in current magnitude (210%; $p<.05$), conductance (183%; $p<.05$), and rate of inactivation (71.4%; $p<.001$), in addition to inducing a hyperpolarizing shift in V50 (−9.402mV; $p<.01$). Future studies will focus on identifying this serum factor in order to evaluate its potential role in nociceptive signal modulation and cellular excitability. Supported in part by NIH grant R21DA034448 (SMT).